Pestic. Sci. 1997, **50**, 195–202

Translocation of Pymetrozine in Plants

Peter Wyss* & Martin Bolsinger

Research Biology Insecticides, Novartis Crop Protection AG, CH-4002 Basel, Switzerland

(Received 15 May 1996; revised version received 15 November 1996; accepted 4 March 1997)

Abstract: Pymetrozine, a pyridine-azomethine, is a selective biocide against aphids, whiteflies and plant hoppers with a high plant systemic activity. By means of bioassays and autoradiographic techniques it has been shown that this systemic behaviour originates not only from xylem but also from phloem mobility. After foliar application the growing points of plants are protected by pymetrozine imports mainly from leaves. This indicates a high importance of phloem mobility for the systemic activity of pymetrozine against plant-sucking insects.

Pestic Sci., **50**, 195–202, 1997
No. of Figures: 5. No. of Tables: 2. No. of Refs: 19

Key words: insecticide, pymetrozine, plant systemic behaviour, phloem mobility

1 INTRODUCTION

Novel insecticides are developed for high selectivity against target pests at low application rates. One of the most important points required to achieve this goal consists in the ability of the compounds to move systemically within a plant. Therefore they have to be mobile in the vascular bundles, i.e. in the xylem and phloem. In the xylem, inorganic ions and, to a certain extent, also organic molecules are translocated acropetally in the transpiration stream from roots to the stem and leaves.[1–3] The translocation of photo-assimilates in the phloem on the other hand occurs in both acropetal and basipetal direction. It is driven by a source-sink mechanism, where the mature autotrophic leaves are the sources and the heterotrophic parts such as roots and young leaves are sinks of carbohydrate.[4–6]

With regard to insecticides, transport in the phloem is of major interest because it enables control at remote locations of a plant such as roots or growing points after foliar application. Models have been created which try to predict the phloem mobility of pesticides on the basis of their physicochemical properties.[7–12] According to these models, phloem mobility depends on a favourable combination of the octanol/water partition coefficient (K_{ow}) and the acid dissociation constant (pK_a), whereas a low log K_{ow} seems to be the most determinant factor enabling non-ionised molecules to be translocated in the phloem. In accordance with these models, pymetrozine, a pyridine-azomethine (Fig. 1) which is largely unchanged at physiological pH, having a pK_a of 3·07, should be phloem-mobile as a consequence of its low log K_{ow} of $-0{\cdot}18$.[13] Pymetrozine is a highly selective biocide against plant-sucking insects, provoking a feeding stop and subsequent starvation to death.[14]

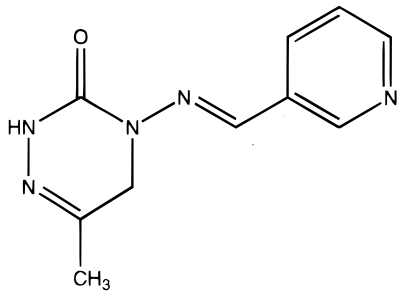

Fig. 1. Pymetrozine: 4,5-dihydro-6-methyl-4-(3-pyridylmethyleneamino)-1,2,4-triazin-3(2*H*)-one.

* To whom correspondence should be addressed.

 Pestic. Sci. 0031-613X/97/$17.50. Printed in Great Britain

Because this compound achieves the best efficacy upon uptake by feeding and much less by direct topical contact, it has to be distributed to the feeding sites in plants to develop a high efficacy.[13] Indeed, soon after its discovery, a good plant systemic action by root uptake was reported.[15] These observations suggest transport in the plant and, at the same time, presence in the phloem, since mainly phloem-sucking insects are affected. The distribution of a compound after root uptake could be explained sufficiently by unidirectional acropetal translocation in the xylem, as it is the most common way for translocation of plant systemic insecticides. The presence of a compound in the phloem does not necessarily mean that it is also translocated in the phloem, since mobility appears to be correlated with the duration of retention of the molecule within the phloem, which depends on the membrane permeability of the compound.[12,16] However, pymetrozine shows a long-term efficacy in the field after foliar application, not only on treated plant parts, but also on leaves which emerge and grow after treatment.[17] The protection of growing points may result from xylem translocation of the compound after absorption by stems and roots. Therefore phloem mobility for pymetrozine could only be hypothesized. For that reason mobility of pymetrozine in plants was investigated using bioassays and autoradiographic techniques.

2 MATERIALS AND METHODS

2.1 Bioassays

2.1.1 *Insects*

Myzus persicae (Sulzer), strain US1L received in 1982 from IACR Rothamsted Experimental Station, UK, was reared on pea seedlings (*Pisum sativum* L. var. Petite Provence from Fenaco, Basel, Switzerland). *Aphis craccivora* (Koch) was collected in 1963 near Aarau, Switzerland, and reared on broadbean seedlings (*Vicia faba* L. var. Witkiens from Samen Mauser, Winterthur, Switzerland). Both strains are standard susceptible.

2.1.2 *Plants*

Tomato (*Lycopersicum esculentum* Mill. var. Marmande from Samen Mauser, Winterthur, Switzerland) and sugar beet plants (*Beta vulgaris* L. *ssp. altissima* var. Reka from FR. Strube, Schoeningen, Germany) were grown in the greenhouse (21°C 14 : 10 h day : night cycles) on peat, consisting of commercially available sphagnum peat + sand (80 + 20 by volume; (pH(H_2O) 6·7; organic matter $>0{\cdot}7$ m^3 m^{-3}). Tomato plants were grown in 300-ml pots and sugar beet plants in 750-ml pots. Six-week-old tomato plants, at the 9–10 leaf stage, and 9 to 11-week-old sugar beet plants, with 11–15 leaves, were used for the experiments.

2.1.3 *Application*

Pymetrozine (Fig. 1) was used as a commercial 250 g kg^{-1} WP (Chess® or Plenum®, Novartis AG, Basel, Switzerland).

For the experiment, five different applications were performed: (I) Plants were treated with pymetrozine in a spray chamber (250 mg AI $litre^{-1}$ 1400 litre ha^{-1}). Soils were either covered with parafilm or not, referred to as shoot or whole plant treatment, respectively. (II) All leaves of a tomato plant were individually treated (250 mg AI $litre^{-1}$) with a De Vilbiss spray pistol. Spraying was stopped just before run-off. Aluminium foil covers and careful spraying prevented contamination of stems and soils. (III) Stems of tomato plants were treated with 5 ml of dispersion (250 mg AI $litre^{-1}$) by painting five 1-ml portions with a brush with a drying period of 1 h in between applications. (IV) Leaflets of tomato plants and whole sugar beet shoots were dipped into 100 ml and 2 litre of pymetrozine dispersions (250 mg AI $litre^{-1}$), respectively and gently agitated for 30 s. The leaves were dried in the same position to prevent contamination of the neighbouring leaflets and the pot soil, respectively. (V) Pot soils were drenched with 20 mg AI $litre^{-1}$ (tomato) and 100 mg AI $litre^{-1}$ (sugar beet) dispersions.

2.1.4 *Sampling and infestation*

Agar (20 g $litre^{-1}$; 2 ml or 5 ml) was poured into Petri dishes (diam. 3·5 cm or diam. 5 cm, respectively). The solidified agar was covered with fresh liquid agar (10–20 g $litre^{-1}$) and punched leaf discs of tomato (diam. 3·5 cm) or sugar beet (diam. 5 cm) were gently placed on the top with adaxial surface (upper side) towards the agar. Aphids of mixed populations were brushed from the appropriate rearing substrate onto leaf discs. Petri dishes were covered with round cotton filters and closed with tight-fitting plastic lids. They were kept in a phytobox (20°C, 12 : 12 h day : night cycles, 60% RH) with the upper leaf side exposed to the light. The virginoparae produced nymphs overnight. Subsequently, the virginoparae, exuviae and any excess nymphs were removed using a vacuum pipe so that 25–40 nymphs remained on each leaf disc.

2.1.5 *Evaluation and statistical analysis*

Mortality was assessed five days after infestation. In all cases, nymphs failing to exhibit repetitive (i.e non-reflex) movement of more than one leg (after gentle prodding if necessary) were scored as dead. Nine replicate leaf discs—three replicate plants and three leaf discs per plant—were used per treatment and concentration. Dif-

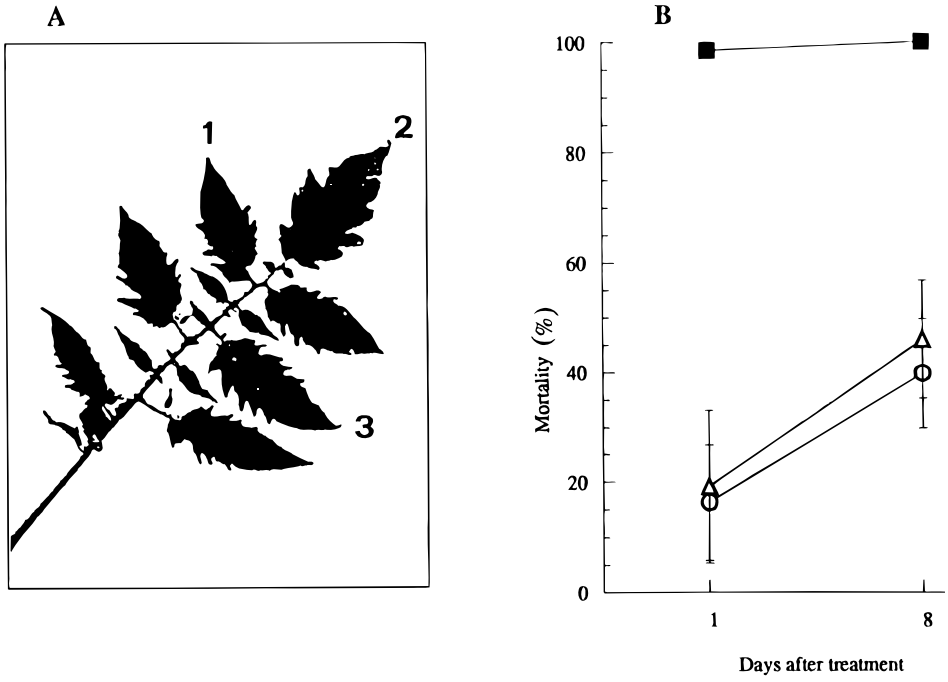

Fig. 2. Mortality of *Myzus persicae* nymphs on tomato leaf after treatment of one leaflet with pymetrozine. A. Positions of (1) treated leaflet and (2 and 3) the two neighbouring leaflets tested. B. Mortality assessed on (■) treated leaflet 1 and on the two neighbouring leaflets (○) 2 and (△) 3. Bars indicate standard error of means.

ferences in insect mortality between treatments were analysed by single factor ANOVA. Assays were repeated twice for consistent results. The results have been corrected for mortality in water-treated controls included in every bioassay.[18] Control mortality was usually below 15%.

2.2 Autoradiography

2.2.1 Test substance

[*Triazine*-6-^{14}C]pymetrozine with an activity of 2·202 MBq mg^{-1} and with a >95% radiochemical purity was used. The radiolabelled compound was formulated as 250 g kg^{-1} WP by grinding the labelled compound and the blank formulation (1 + 3 by weight, 10 min) in an Esco Typ 50/35 mortar (Esco Labor, Riehen, Switzerland).

2.2.2 Leaf treatment

(I) On each of three sugar beet plants, one fully expanded leaf was sandwiched between two pieces of cardboard (10·5 × 7·5 cm). Into the cardboard, facing the upper leaf side, a hole (diam. 5 cm) had previously been punched. The cardboard pieces were clamped together and the leaves were aligned horizontally on support devices. To the exposed leaf disc, bordered by the cardboard on the upper leaf side, small droplets of a 250-ng AI μl^{-1} dispersion were pipetted up to a total volume of 300 μl.

(II) One leaflet of a tomato plant was fixed in a Petri dish (diam. 5 cm), held in a horizontal position by support devices. On the upper leaflet side, small droplets of a 250 ng AI μl^{-1} dispersion were pipetted up to a total volume of 300 μl.

Treated leaves and two or three of the youngest leaves of sugar beet plants were harvested four hours, three days and six days after treatment. The tomato leaf with the treated leaflet was harvested six days after treatment. Surface deposits were washed off by shaking treated leaves in water + methanol (1 + 1 by volume; sugar beet, 500 ml; tomato leaflet, 50 ml; 2 × 1 min). Leaves of both plants were immediately pressed between filter paper, prohibiting long-distance translocation. After two weeks the plant material was mounted on 40 × 20 cm cardboard and covered with cellophane foil.

2.2.3 Stem application of tomato plants

A 5-ml plastic pipette tip was cut up longitudinally and braced around the stem between leaves 3 and 4. The plastic tip was fixed and sealed using parafilm. To guarantee 100% sealing the tip was wrapped additionally with a cotton swab. A volume (700 μl) of a 150-ng AI μl^{-1} dispersion was pipetted into the tip. After 40 h exposure, roots were washed and the whole plant was pressed between filter paper. After 2 weeks the plant was cut into five parts, which were mounted on 40 × 20 cm cardboard and covered with cellopane foil.

2.2.4 Autoradiography

Autoradiography was performed using a Fujibas® 1000 bio imaging analyser system (Fuji, Japan). Fujibas®

1000 imaging plates were exposed to the mounted plant material and subsequently scanned by a Fujibas® 1000 scanner. Data were transferred with BAS Reader 2.6® software (Fuji, Japan) to a personal computer and hard copies were generated by data conversion with TINA 2.08® (Raytest, Germany).

3 RESULTS

3.1 Bioassays

3.1.1 Tomato

Dipping of a tomato leaflet into a pymetrozine 250 g kg^{-1} dispersion at 250 mg AI $litre^{-1}$ resulted, at one day after treatment, in 100% mortality of *M. persicae* on the treated leaflet and in 16 to 20% mortality on neighbouring leaflets (Fig. 2). Mortality on neighbouring leaflets increased to between 39 and 46% at eight days after treatment. Mortality rates on neighbouring leaflets were found to be independent of leaflet position ($P > 0{\cdot}6$). Spray and drench application also resulted in high levels of mortality of *M. persicae* three weeks after application (Table 1). Drench application to soil with pymetrozine (20 mg AI $litre^{-1}$) resulted in 97% mortality of *M. persicae* on leaves which were already fully expanded at the time of treatment and in 77% mortality on leaves which emerged and grew after the treatment. After spray application (250 mg AI $litre^{-1}$) of whole tomato plants, 100% mortality of *M. persicae* was found on leaves which were already fully expanded at the time of treatment. In addition, activity against *M. persicae* was also found on leaves which emerged and developed after treatment. Interestingly, the mortality rates evaluated on these leaves did not differ between the various modes of spray application, i.e. whole plant, shoot or leaves ($P > 0{\cdot}25$) (Table 1). A somewhat weaker but still clear effect against *M. persicae* was observed at three weeks after stem treatment with pymetrozine (5 ml of 250 μg AI ml^{-1}). Mortality was assessed at 45% on leaves which were fully expanded at the time of treatment and 25% on leaves which emerged and grew after treatment.

3.1.2 Sugar beet

Drench and spray application to sugar beet plants resulted in a high efficacy against *A. craccivora* (Table 2). After 100 μg ml^{-1} pymetrozine drench application, a

TABLE 1
Efficacy of Pymetrozine against Nymphs of *Myzus persicae* after Application to Various Parts of Tomato Plants

Application of pymetrozine			*Mortality of* Myzus persicae *on leaves*	
Plant part treated	*Mode of application*	*Conc. (mg AI litre^{-1})*	*Fully expanded at treatment time* (%) ($\pm SE$)	*Emerged after treatment* (%) ($\pm SE$)
Whole plant	Spray	250	100 ($\pm$0)	49 ($\pm$8·3)
Shoot	Spray	250	99 ($\pm$0·6)	57 ($\pm$9·6)
Leaves	Spray	250	100 ($\pm$0)	41 ($\pm$6·7)
Root	Soil drench[a]	20	97 ($\pm$1·7)	77 ($\pm$4·2)
Stem	Painting[b]	250	45 ($\pm$8·6)	25 ($\pm$10·6)

[a] 300 ml pot volume.
[b] 5 ml.

TABLE 2
Efficacy of Pymetrozine against Nymphs of *Aphis craccivora* after Application to Various Parts of Sugar Beet Plants

Application of pymetrozine			*Mortality of* Aphis craccivora *on leaves*	
Plant part treated	*Mode of application*	*Conc. (mg AI litre^{-1})*	*Fully expanded at treatment time* (%) ($\pm SE$)	*Emerged after treatment* (%) ($\pm SE$)
Whole plant	Spray	250	100 ($\pm$0)	34·6 ($\pm$11·0)
Leaves	Spray	250	100 ($\pm$0)	30·0 ($\pm$7·8)
Leaves	Dip	250	100 ($\pm$0)	32·9 ($\pm$10·9)
Root	Soil drench[a]	100	97 ($\pm$1·7)	78·8 ($\pm$10·6)

[a] 750 ml pot volume.

mortality of 97% was assessed on leaves which were already fully expanded at the time of treatment, and of 78% on leaves which emerged and grew after treatment. After foliar and dip application at 250 μg AI ml^{-1} a mortality of 100% was found on leaves which were fully expanded at the time of treatment. Post-treatment emerged leaves showed 30 to 34% mortality on *A. craccivora*. As on tomato plants, mortality rates on these leaves did not depend on the mode of application, i.e. foliar treatment of the whole plant or only the leaves, or dipping of the leaves gave similar results ($P > 0{\cdot}6$) (Table 2).

In all bioassays, insects affected by pymetrozine showed the same symptoms whether they were exposed to treated leaves, to leaves grown after foliar treatment, to leaves after drench or stem application or to untreated leaflets neighbouring a treated one. They kept walking continuously and obviously lost weight. Within two to four days after infestation they died.

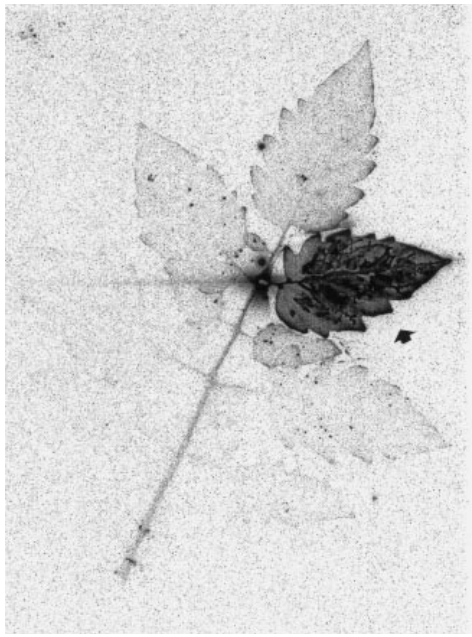

Fig. 3. Autoradiograph of a tomato leaf six days after [^{14}C]pymetrozine treatment of a leaflet (arrow). Deposits on the treated leaflet were washed off.

3.2 Autoradiography

Six days after treatment of a tomato leaflet with [^{14}C]pymetrozine, the radiolabel was distributed over the whole leaf (Fig. 3). In contrast, in the petiole and in the main leaf vein, the label is already very well distributed a few hours after application (results not shown). Comparable autoradiographs were obtained upon leaf treatment of sugar beet plants (Fig. 4). The label was well distributed a few hours after application in the

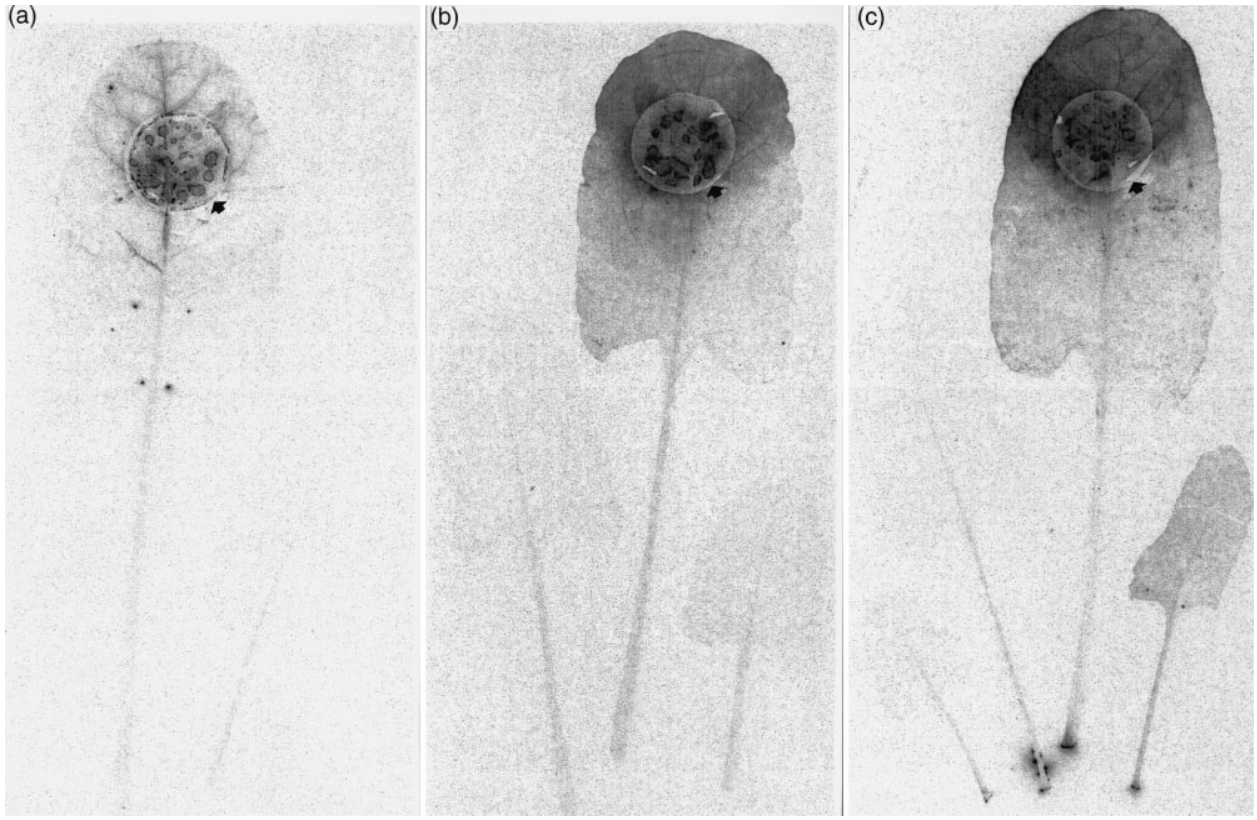

Fig. 4. Autoradiographs of sugar beet leaves (a) 4 h, (b) three days and (c) six days after [^{14}C]pymetrozine treatment of one leaf disc (arrow) per plant. Deposits on treated leaves were washed off.

treated leaf and appeared not only in the petiole of treated leaves but also in untreated young leaves. Over the following days it accumulated in untreated young leaves. In contrast to treated leaves, with an increasing density of the label towards the apical points, an equal distribution occurred in the untreated leaves. Similar distribution patterns could be observed in tomato plants after stem application (Figs 5(a) and 5(b)). The label was distributed over the whole plant, whereas the pattern was more dense in leaves above the treatment site, i.e. in apicalar direction. However, the label was found as well in leaves below the treatment site and in

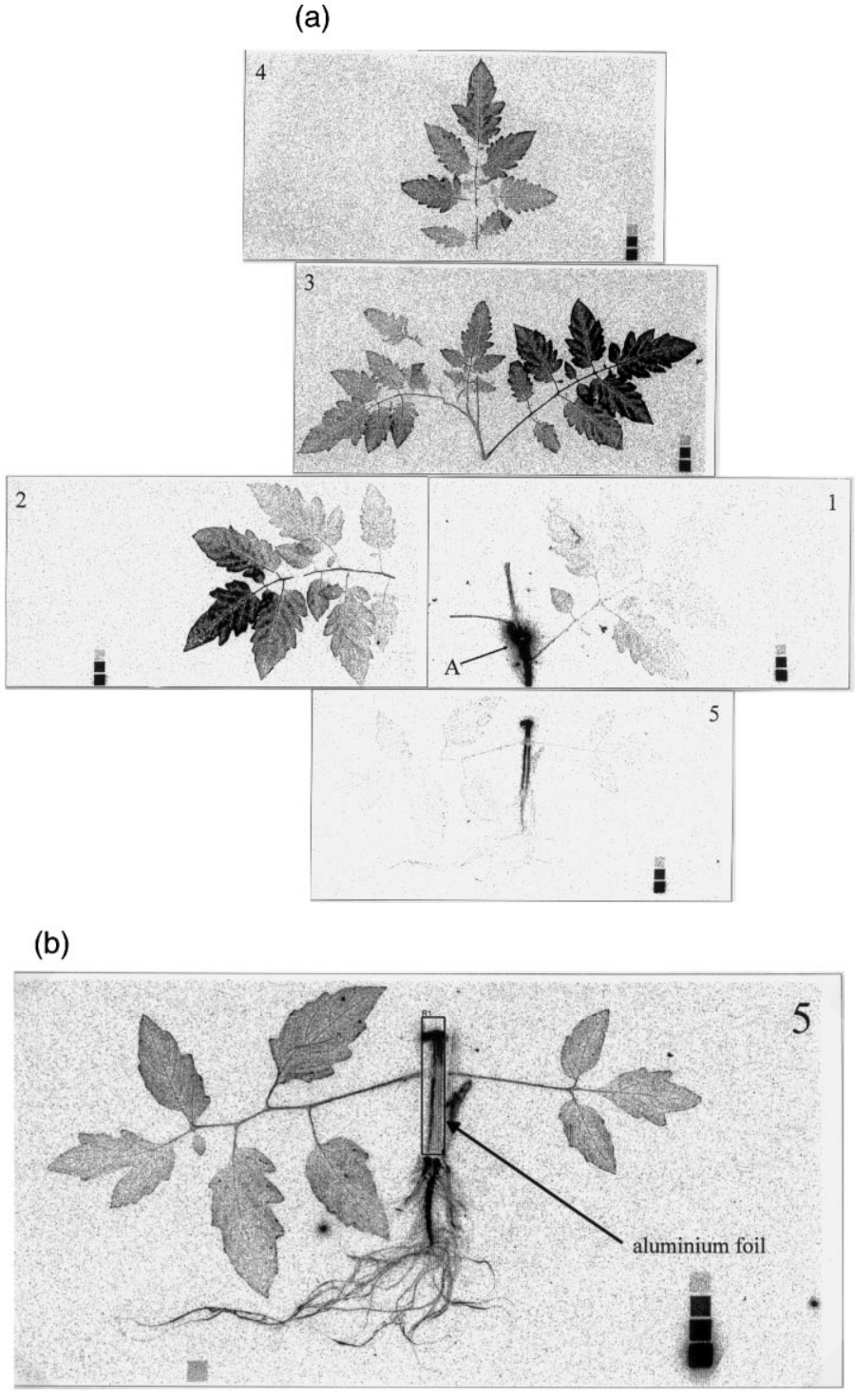

Fig. 5. Autoradiographs of a tomato plant 40 h after application of [^{14}C]pymetrozine to the internode between leaves 3 and 4, indicated by the letter A. (a) Imaging plates (1–5) were exposed 2 h. (The arrangement of the plates shows the partitioning of the plant into five pieces.) (b) Imaging plate 5 was exposed to the part below the application site for 18 h. The stem below the application site was covered with an aluminium foil for shielding to overexposure.

the whole root system up to the tips of the finest roots (Fig. 5(b)).

4 DISCUSSION

Pymetrozine provokes an inhibition of stylet penetration and a subsequent starvation to death of plant-sucking insects. These effects can be observed either after topical application on insects or after feeding uptake on treated plants.[14] In our bioassays, these symptoms were confirmed on *M. persicae* and *A. craccivora.* Moreover, aphids showed the same behaviour on leaves emerged and developed after foliar treatment as on untreated tomato leaflets neighbouring a treated one. From the fact that metabolism products of pymetrozine in plants do not show insecticidal activity (Szczepanski, H., 1995, pers. comm.), it can be concluded that the observed plant systemic activity is a result of parent pymetrozine which was translocated to the site of activity. The high efficacy of pymetrozine after drench application suggests a good mobility from root to shoot in the xylem.[1–3] This acropetal translocation was also observed on autoradiographs after foliar [^{14}C]pymetrozine application with an increasing radioactivity towards the shoot and leaf tips.

However, not all of the phenomena observed in the bioassays can be explained by acropetal translocation. The efficacy assessed in bioassays (I) on untreated tomato leaflets in a basipetal position to a treated leaflet on the same leaf, (II) on leaves grown after spray application of tomato leaves and (III) on leaves of sugar beet plants grown after shoot dip application, is the result of an export of the compound from treated to untreated leaves. Export of a compound from leaves occurs under normal conditions exclusively in the phloem.[6] Basipetal translocation of pymetrozine was also evident on autoradiographs. In tomato plants, for example, the label could be detected in the fine roots after stem application. Basipetal distribution of label was further observed after leaf application. Within four hours after [^{14}C]pymetrozine application to sugar beet leaves, radioactivity could be localized in the petiole of the treated leaf and in petioles of young leaves. The label then gradually accumulated and became equally spread in young leaves during the next few days. No density gradient towards the leaf edges, indicating a xylem translocation, could be observed in these leaves. Remarkably, the degree of accumulation and hence the import rate depends on leaf development stage, with the highest rate in the youngest leaf. This is another indication of the phloem mobility of pymetrozine, since the strongest sink for assimilates is created by emerging leaves. In sugar beet plants maximal import occurs at approximately 20% full leaf length.[19] When leaves are 30 to 60% fully expanded, they stop importing and begin to export.[5] The distribution of pymetrozine reflects therefore exactly the translocation pattern of photosynthates and it seems likely that the compound moves out from the treated leaf as well as into the untreated young leaves in the phloem.

The experiments performed suggest, therefore, that pymetrozine is phloem-mobile. This finding is supported also by models which predict phloem mobility for non-ionised molecules with a low $\log K_{ow}$.[7–12] Pymetrozine has a $\log K_{ow}$ of $-0{\cdot}18$.[13] However the density shown in autoradiographs is much weaker in basipetal than in acropetal direction of the application site, indicating much better xylem than phloem mobility. In spite of this relationship it seems that phloem translocation, especially export from leaves, plays an important role in the systemic behaviour of the compound. Foliar application of whole plants and spray application of leaves resulted in the same efficacy against the test insects on leaves grown after treatment. Pymetrozine taken up by the stem or by the roots through the soil after foliar application may therefore be neglected in the contribution to the observed systemic effects.

ACKNOWLEDGEMENTS

We wish to thank Dr H. Buholzer, Dr C. Flückiger, Dr G. Nicollier, Dr P. Sandmeier, M. Schaeuble, R. Senn, Dr A. Steinemann and A. Stieger of Novartis AG, Basel, for their scientific and technical support.

REFERENCES

1. Canny, M. J., Apoplastic water and solute movement: New rules for an old space. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **46** (1995) 215–36.
2. Boyer, J. S., Water transport. *Ann. Rev. Plant Physiol.*, **36** (1985) 473–516.
3. Bollard, E. G., Transport in the xylem. *Ann. Rev. Plant Physiol.*, **11** (1960) 141–66.
4. Bel, A. J. E., The transport phloem. Specifics of its functioning. In *Progress in Botany*, ed. H. D. Behnke, U. Lüttge, K. Esser, J. W. Kadereit & M. Runge. Springer-Verlag, Berlin, 1993, pp. 134–50.
5. Turgeon, R., The sink-source transition in leaves. *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, **40** (1989) 119–38.
6. Cronshaw, J., Lucas, W. J. & Giaquinta, R. T., eds, *Plant Biology, Vol. 1: Phloem transport*. Liss, New York, 1986, 650 pp.
7. Bromilow, R. H., Chamberlain, K. & Evans, A. A., Molecular structure and properties of xenobiotics in relation to phloem translocation. In *Recent Advances in Phloem Transport and Assimilate Compartmentation*, ed. J. L. Bonnemain, S. Delrot, W. J. Lucas & J. Dainty. Ouest Editions, Nantes, France, 1991, pp. 332–40.
8. Bromilow, R. H., Rigitano, R. L. O., Briggs, G. G. & Chamberlain, K, Phloem translocation of non-ionised chemicals in *Ricinus communis. Pestic. Sci.*, **19** (1987) 85–99.

9. Grayson, B. T. & Kleier, D. A., Phloem mobility of xenobiotics. IV. Modelling of pesticide movement in plants. *Pestic. Sci.*, **30** (1990) 67–9.
10. Kleier, D. A., Phloem mobility of xenobiotics. I. Mathematical model unifying the weak acid and intermediate permeability theories. *Plant Physiol.*, **86** (1988) 803–10.
11. Kleier, D. A., Phloem mobility of xenobiotics. V. Structural requirements for phloem-systemic pesticides. *Pestic. Sci.*, **42** (1994) 1–11.
12. Tyree, M. T., Peterson, C. A. & Edington, L. V., A simple theory regarding ambimobility of xenobiotics with special reference to the nematicide oxamil. *Plant Physiol.*, **63** (1979) 367–74.
13. Tomlin, C. (ed.) *The Pesticide Manual, 10th edn.* The British Crop Protection Council and The Royal Society of Chemistry, Surrey and Cambridge, 1994, p. 868.
14. Harrewijn, P. & Kayser, H. K., Pymetrozine, a fast-acting and selective inhibitor of aphid feeding. *In-situ* studies with electronic monitoring of feeding behaviour. *Pestic. Sci.*, **49** (1997) 130–40.
15. Flückiger, C., Kristinsson, H., Senn, R., Rindlisbacher, A., Buholzer, H. & Voss, G., CGA 215′944—a novel agent to control aphids and whiteflies. *Brighton Crop Prot. Conf., Pests and Diseases*, 1992, pp. 43–50.
16. Jachetta, J. J., Appleby, A. P. & Boersma, L., Apoplastic and symplastic pathways of atrazine and glyphosate transport in shoots of seedling sunflower. *Plant Physiol.*, **82** (1986) 1000–7.
17. Follas, G., Evaluation of pymetrozine to control green peach aphid on stonefruit. *Proc. 46th N.Z. Plant Protection Conf.*, pp. 1993, 21–3.
18. Abbott, W. S., A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.*, **18** (1925) 265–7.
19. Fellows, R. J. & Geiger, D. R., Structural and physiological changes in sugar beet leaves during sink to source. *Plant Physiol.*, **54** (1974) 877–85.